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Expression of High Amounts of the CD117 Molecule in a Case of B-Cell Non-Hodgkin's Lymphoma Carrying the t(14:18) Translocation

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The c-kit proto-oncogen (CD117) has been described to be present in normal and neoplastic hemopoietic cells including both myeloid and lymphoid lineages. Among the normal lymphoid cells CD117 expression would be restricted to a small subset of NK-cells, and to early T-cell precursors and it is not expressed by normal B-cells. Regarding chronic lymphoproliferative disorders the only data provided up to now suggests that CD117 expression is restricted to cases of Hodgkin's disease and anaplastic large-cell lymphoma. In the present paper we describe a case of a B-cell chronic lymphoproliferative disorder carrying the t(14:18) translocation as demonstrated by molecular studies, in which the flow cytometric immunophenotypic analysis of both peripheral blood and bone marrow samples revealed the expression of high amounts of the CD117 antigen in the surface of the clonal B-cell population. Further studies are necessary to explore both the functional role of c-kit expression in the neoplastic B-cells from this patient and its potential utility for the diagnosis and follow-up of patients with B-cell non-Hodgkin's lymphoma. Am. J. Hematol. 63:226–229, 2000.

Key words: CD117; bcl-2; flow cytometry; immunophenotype; lymphoma

INTRODUCTION

The c-kit proto-oncogene (CD117) encodes a transmembrane tyrosine kinase receptor [1,2] which is expressed in several cell types, including normal and neoplastic hematopoietic cells (reviewed in [3]). CD117 expression can be detected in both normal myeloid and lymphoid lineages, although for this latter lineage it would be restricted to a small NK-cell subset which coexpresses $CD56^{bright}$ and both the high- and intermediate-affinity IL-2 receptors [4], and to early T-cell precursors [5]. Moreover, it is known that interactions between c-kit and stem cell factor (SCF) play important roles in the generation of several hematopoietic lineages [6] although it has been recently suggested that B-cell development can occur normally in the absence of c-kit [7].

Among hematological malignancies, CD117 expression was initially associated with acute myeloblastic leukemia (AML) [8–15]. Nevertheless, it is now well established that it may also be found in a relatively important proportion of T-acute lymphoblastic leukemia (ALL) while it is usually absent in B-lineage ALL [15–18]. Recently, it has also been reported that in multiple myeloma (MM) cases and patients with monoclonal gammopathy of undetermined significance (MGUS) clonal plasma cells can display reactivity for CD117 in around one-third of the patients [19,20] while plasma cells from normal bone marrow (BM) samples are constantly CD117 negative.

In spite of this, few information has been reported regarding CD117 expression in human lymphomas, in-

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cluding few cases of Hodgkin's disease (HD) and anaplastic large cell lymphoma (ALCL) [21].

Herein we report a case of a chronic lymphoproliferative disorder carrying the t(14:18) translocation, in which the immunophenotypic studies revealed the presence of high amounts of CD117 in the surface of the clonal B-cell population.

PATIENTS AND METHODS

Case Report

A 71-year-old man was referred for evaluation at the Haematology Service of the Hospital Ramón y Cajal (Madrid, Spain) due to the presence of an absolute lymphocytosis in a routine haematological analysis. The patient was asymptomatic, and the only finding on physical examination was the presence of enlarged lymph nodes in the cervical and the axillary regions. CT scan revealed the presence of an enlarged spleen (13 mm) and multiple lymphadenopathies involving the axillar, mediastinic, mesenteric, periaortic, and iliac nodes.

Peripheral blood (PB) cell counts were as follows: hemoglobin (Hb) 15 g/dL; platelets 150×10^9/L; leukocyte count 99.1×10^9/L with 4.53% neutrophils, 91.2% lymphocytes, 2.89% monocytes, 0.8% eosinophils, and 0.4% basophils. The PB smears stained with May–Grünwald–Giemsa showed the presence of a large number of lymphocytes most of which (70%) were relatively small, with scanty cytoplasm and a round nucleus with clumping chromatin; the remaining lymphocytes (30%) were larger and displayed an excentric nucleous, a prominent nucleolus, and a more basophilic and larger cytoplasm.

Lactic dehydrogenase (LDH) and β-2 microglobulin serum levels were 598 units/L and 2.6 mg/L, respectively. Serum immunoglobulin levels as well as the other parameters explored were within the normal range.

In the BM aspirate an infiltration by lymphocytes which displayed the same morphological features as those described for peripheral blood was detected. A marked mast cell hyperplasia was also observed.

BM biopsy showed the presence of focal and interstitial infiltrates of small lymphocytes with a high nuclear–cytoplasmic ratio, condensed chromatin, and inconspicuous nucleoli. In the areas with focal infiltrates prolymphocytes with less-condensed chromatin and a clear nucleolus were frequently observed. Immunohistochemical analysis of the bone marrow showed the co-expression of CD20, CD5, and bcl-2 in the neoplastic cells. Lymph node biopsy could not be performed because the patient refused to undergo the procedure.

Immunological Markers Analysis

Immunophenotypic analysis was performed in erythrocyte-lysed whole PB and BM samples by means of a stain and then lyse protocol. Antigen expression was analyzed by direct immunofluorescence using either triple- or double-staining combinations of monoclonal antibodies directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), and either peridin chlorophyll protein (PerCP) or PE/cyanin 5 (Cy5) fluorochrome tandem. The following monoclonal antibody conjugates were used: (1) FITC conjugated anti CD5, CD10, CD16, CD18, CD19, CD44, CD45RA, CD69, κ light chain (purchased from Becton-Dickinson, San Jose, CA), CD7, CD11a, CD11b, CD11c, CD15 CD40, CD65 (Caltag Laboratories, San Francisco, CA), CD35, β-2 microglobulin (Cymbus, Biosciences, Ltd., Southampton, UK), CD43, CD49d, CD49e (Serotec, Oxford, UK), CD51, FMC7 (Immunotech, Marseille, France), CD54 (CLB, Amsterdam, The Netherlands), CD103, bcl-2 (Dakopatts, Glostrup, Denmark), CD117, and CD138 (IMICO, Madrid, Spain); (2) PE conjugated anti CD2, CD3, CD4, CD13, CD14, CD19, CD22, CD23, CD33, CD34, CD45RO, λ light chain (purchased from Becton-Dickinson, San Jose, CA), CD5 (Caltag Laboratories, San Francisco, CA), CD25, CD56 (Coulter Corporation, Miami, FL), CD79b (Immunotech, Marseille, France), CD117 (IMICO, Madrid, Spain), and CD123 (Pharmingen, San Diego, CA); (3) PE/Cy5-conjugated anti-CD20, CD45 (Becton-Dickinson, San Jose, CA), CD19, and CD38 (Caltag Laboratories, San Francisco, CA).

Staining of the intracellular bcl-2 protein was performed using the Fix & Perm reagent (Caltag Laboratories, San Francisco, CA) strictly following the instructions of the manufacturer after surface staining for CD19.

Measurements were performed on a FACSCalibur flow cytometer (Becton Dickinson), using the CellQuest software program (Becton Dickinson) for data acquisition and the Paint-A-Gate PRO software program (Becton Dickinson) for further data analysis.

DNA Preparation and Southern Blot (SB) Analysis

High molecular weight DNA was isolated from the bone marrow sample by standard proteinase K digestion, phenol–chloroform extraction, and ethanol precipitation. Southern blot analysis was carried out after digestion with the *Eco*RI, *Bam*HI, *Hin*dIII, *Bgl*II, and *Xba*I restriction enzymes, size fractionated, transferred, and hybridized with ^{32}P-labeled probes as previously described [22]. TcR-β, IgH, Bcl-1, Bcl-2, Bcl-6, c-myc, MLL, p53, and p16 genes were studied using this methodology with the following probes: Cβ1 [23], JH6, MTC, PFL1, PFL2, pB16, Bc16, cD1A, 11q23/MLL, cDNA/p16, and cDNA/p53 [22], respectively.

Polymerase Chain Reaction (PCR) Analysis of the t(14:18) Translocation

The amplification of the breakpoint regions of the translocation was performed by Nested-PCR according

to previously described methods [22]. PCR products from the final reactions were analyzed by electrophoresis in agarose and visualized by staining with ethidium bromide [22]. Both a negative (sterile distilled water and normal DNA) and a positive control (genomic DNA from a positive patient) were used.

RESULTS

The flow cytometric analysis of both PB and BM nucleated cells revealed the presence of a clonal expansion of B lymphocytes which represented 68% and 65% of the total cellularity, respectively, with strong λ-light chain expression in the cell surface. Additionally, both BM and PB clonal B cells showed strong reactivity for the CD19, CD22, CD40, CD45, CD45RA, CD49d, CD117 (Figure 1), CD123, and β-2 microglobulin antigens; they expressed the CD5, CD18, CD23, CD35, CD38, CD43, CD138, and FMC7 molecules at a lower intensity, and they were negative for the CD2, CD3, CD4, CD7, CD10, CD11a, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD44, CD45RO, CD49e, CD51, CD54, CD56, CD65, and CD69 antigens.

Over-expression of the bcl-2 protein was found both in BM and PB clonal B-cells from this patient (Figure 1C).

Molecular studies performed on the BM sample showed the presence of bcl-2 gene rearrangements. PCR analysis revealed the existence of amplification of the t(14:18) breakpoint region. No molecular rearrangements were found for the other gene sequences analyzed.

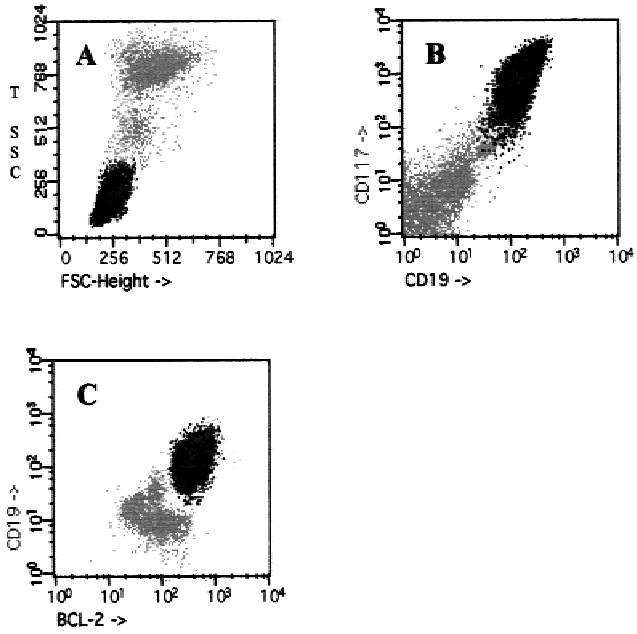

Fig. 1. Representative dot plots of the light scatter pattern (FSC/SSC) and the immunophenotypical characteristics of clonal CD19⁺ B-cells (black dots). As shown in panel A, clonal B-cells display a FSC/SSC distribution similar to normal residual lymphocytes. CD117 and bcl-2 expression by clonal CD19⁺ B-cells is shown in panels B and C, respectively.

DISCUSSION

The case reported here shows the unusual expression of high levels of the CD117 antigen in the surface of a population of clonal B-cells in a case of B-cell non-Hodgkin's lymphoma carrying the t(14:18) translocation.

Using both immunostainings and the analysis of CD117 RNA expression by Northern blot hybridization, expression of c-kit has been reported in 11 out of 21 Hodgkin's disease cases and in 11 out of 16 $CD30^+$ anaplastic large-cell lymphomas, 4 of them with a T-cell immunophenotype [21]. In contrast, c-kit expression has been reported as negative in other subtypes of NHL such as small lymphocytic lymphoma, follicular lymphoma, diffuse small- and large-cell NHL, large-cell immunoblastic NHL, small non-cleaved NHL, and mycosis fungoides [21]. To the best of our knowledge, this is the first report in which a case of NHL with the t(14:18) translocation expressing the c-kit molecule is described. Regarding B-cell ALL, expression of c-kit was initially reported as negative [8,24]; nevertheless, expression of c-kit was recently described in 34 (4%) out of 819 ALL cases [18]. From these patients, two-thirds were classified as having T-cell ALL lineage (mainly of the pro-T-ALL or T-1 subtype), and one-third as precursor-B-ALL; overall these cases represented 11% and 2% of all T- and precursor-B-ALL cases, respectively. Interestingly, cells from 62% of these ALL cases co-expressed other myeloid markers (CD13, CD33, or both). These findings in ALL cases together with the expression of CD117 in acute undifferentiated leukemias suggest that the reactivity for c-kit may identify a subgroup of cases, which may correspond to leukemias either arising from early prothymocytes and/or early hematopoietic cells, both being able to differentiate into the lymphoid and myeloid cell lineages [18]. However, it should be noted that the expression of CD117 has also been reported in mature-appearing neoplastic B-cells from other hematological malignancies [3,10,25–28].

Preliminary studies have shown that early B-cell precursors in mouse bone marrow may constitutively express the c-kit antigen [29]. However, more recently immunophenotypic studies performed on $CD19^+$ B-cells from normal human bone marrow showed the absence of any subpopulation of normal B-cells co-expressing the CD19 and CD117 antigens at least to a frequency higher than one cell in one hundred thousand cells (10^{-5}) [30]. Moreover, in bone marrow samples of more than 50 patients suffering from B-cell chronic lymphoproliferative disorders with bone marrow involvement studied in our laboratory, including chronic lymphocitic leukemia and

Waldenstrom's macroglobulinemia, the expression of the CD117 molecule in clonal B-cell population has not been found (data not shown). On the basis of these results, the presence of reactivity for the CD117 antigen in B-cell chronic lymphoproliferative disorders could be considered as an "aberrant phenotype" [19]. Whether this marker could be associated with specific morphological, histological, phenotypic, or chromosomal features in B-cell non-Hodgkin's lymphomas remains unclear.

Previous studies have shown that the SCF/c-kit interaction plays a crucial role on hematopoietic stem cells [6]; however, more recently it has been suggested that signalling through the SCF receptor (CD117) is not essential for the in vivo development of B-cells [7,29,31].

In this sense, it should be noted that further studies are necessary in order not only to identify other cases expressing c-kit antigen but also to establish a functional role of CD117 expression in B-cell chronic lymphoproliferative disorders and its potential utility for the diagnosis and follow-up of minimal residual disease of certain subtypes of B-cell NHL.

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